

Antibody Responses of Raccoons Naturally Exposed to Influenza A Virus

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Abstract

An investigation was performed to describe the responses of naturally acquired antibodies to influenza A virus in raccoons (*Procyon lotor*) over time. Seven wild raccoons, some of which had been exposed to multiple subtypes of influenza A virus, were held in captivity for 279 days, and serum samples were collected on 10 occasions during this interval. Serum samples from 9 of 10 bleeding occasions were tested using an epitope-blocking enzyme-linked immunosorbent assay for the presence of antibodies to influenza A virus. Although titer declines were noted in most animals over time, all animals maintained detectable antibodies for the duration of the study. These data indicate that naturally acquired antibodies to influenza A virus can remain detectable in raccoons for many months, with the actual duration presumably being much longer because all animals had been exposed to influenza A virus before this study commenced. This information is important to surveillance programs because the duration of naturally acquired antibodies to influenza A virus in wildlife populations is largely unknown.

Key Words: Antibody—Influenza A virus—*Procyon lotor*—Raccoon.

INFLUENZA A VIRUSES ARE CLASSIFIED into different subtypes based on the antigenicity of the hemagglutinin (HA) and neuraminidase (NA) proteins (Spackman 2008). To date, 16 HA subtypes (H1–H16) and 9 NA subtypes (N1–N9) have been identified (Fouchier et al. 2005). These viruses are often further subdivided into strains associated with specific hosts (e.g., avian, equine, swine, and human influenza viruses). At present, most genetic and biologic data of these viruses have been obtained from domestic animals; information on wild and feral species is limited (Webby et al. 2007). Therefore, a thorough understanding of the epidemiology of these viruses in wildlife populations is a high priority for future management decisions because a high percentage of emerging zoonotic diseases are wildlife-borne (Taylor et al. 2001).

Evidence of natural influenza A virus exposure (i.e., antibodies or active infection) has been reported in a variety of wild and captive mammalian species (VanDalen et al. 2009). For example, evidence of natural exposure has been obtained in the Old World from captive mink (*Mustela vison*) (Klingeborn et al. 1985), stone marten (*Martes foina*) (WHO 2006), Otswon's civet (*Chrotogale owstoni*) (Robertson et al. 2006), captive tigers (*Panther tigris*) (Thanawongnuwech et al. 2005), and captive leopards (*P. pardus*) (Keawcharoen et al.

2004). In the New World, evidence of natural exposure to influenza A viruses is more limited in wild mammals. Nonetheless, some of the first evidence of serious disease in wild mammals was obtained from harbor seals (*Phoca vitulina*) (Geraci et al. 1982). In addition, antibodies to influenza A virus have been detected in raccoons (*Procyon lotor*) and feral swine (*Sus scrofa*) in the United States (Hall et al. 2008a, 2008b). However, the duration of these antibodies in many wildlife species has yet to be fully examined.

Limited studies have recently been initiated to gain an understanding of the significance of wild mammals in the epidemiology of select subtypes of influenza A virus. For example, it has been shown that experimentally infected red foxes (*Vulpes vulpes*) shed the highly pathogenic H5N1 subtype (Reperant et al. 2008), and there is evidence that raccoons can become infected with and can transmit various subtypes of influenza A virus (Hall et al. 2008a).

Estimation of the persistence of influenza A virus antibodies in naturally exposed subjects has been largely neglected. However, it has been noted that a detectable antibody response in naturally exposed humans was long lasting, up to nearly 5 years in patients followed that long (Kitphati et al. 2009). In addition, following a natural infection of influenza A

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TABLE 1. SUMMARY OF ANTIBODY RESPONSES OF RACCOONS NATURALLY EXPOSED TO VARIOUS SUBTYPES OF INFLUENZA A VIRUS

Raccoon	Time (days) ^a									Subtype ^b
	1–13	25	57	124	176	206	236	263	279	
1	nt	640 ^c	640	40	80	80	80	80	nt	H1+H3+H4N2+N6
2	80	80	80	40	nt	40	40	40	40	H1+H4N6
3	80	80	40	40	40	40	20	40	40	H4N6
5	160	80	160	40	40	40	40	40	40	H4N6
12	40	160	160	160	nt	160	160	80	80	H3+H6N8
15	2560	nt	1280	1280	nt	1280	1280	640	640	H4N6+N7
16	40	nt	80	80	20	20	20	20	20	H3+H6N8

^aTime point of sequential bleedings following capture.

^bSubtype of influenza A virus associated with the detected antibodies as determined by hemagglutination-inhibition and neuraminidase-inhibition tests with equine red blood cells (RBCs) at the National Veterinary Services laboratory. This test has not been validated by National Veterinary Services Laboratory; however, compared with bELISA data it appears to perform better for raccoon sera than standard chicken RBC assays. H10, H13, and H15 were not tested using equine RBCs. All subtypes were associated with time point three (i.e., day 57).

^cAntibody titer as determined by serial dilutions in bELISAs (see text).

H, hemagglutinin; N, neuraminidase; bELISA, epitope-blocking enzyme-linked immunosorbent assay; nt, not tested due to insufficient serum volume.

virus in swine (e.g., swine influenza virus: H3N2), antibodies to this virus could still be detected 28 months postinfection (Desrosiers et al. 2004). The scientific literature is surprisingly limited on the long-term persistence of select influenza A virus antibodies following natural infections (Desrosiers et al. 2004), especially in wildlife species.

Twenty-two raccoons were captured in Larimer County, CO, during the spring of 2006. The husbandry and handling procedures for 20 of these animals are described elsewhere (Root et al. 2008). Eight (36%) raccoons yielded evidence of influenza A virus exposure at their time of capture using an epitope-blocking enzyme-linked immunosorbent assay (bELISA) (Sullivan et al. 2009). Seven of eight antibody-positive raccoons (five females and two males) were held in captivity and serially bled over time (i.e., up to 279 days). The body mass of the antibody-positive raccoons ranged from 6.5 to 9 kg.

The captive raccoons were sampled periodically on 10 occasions over the 279-day period (Table 1). However, sufficient serum volume for serology was only available from nine occasions. Using the aforementioned bELISA, sera were tested at multiple dilutions (1:10–1:5120) for the presence of antibodies to influenza A virus. Sera from one bleeding occasion (time point 3, day 57; see Table 1) were sent to the National Veterinary Services Laboratory (Ames, IA) and subtyped using modified versions (e.g., the use of horse erythrocytes for most subtypes) of the standard hemagglutination-inhibition and NA-inhibition protocol (Killian 2008). This test has not been validated by National Veterinary Services Laboratory, but compared with bELISA data, it appears to perform better for raccoon sera than standard chicken red blood cell (RBC) assays.

Results suggested that raccoons maintain a detectable serum antibody response for long periods of time following natural exposure to select subtypes of influenza A virus (Table 1). The raccoons in this study were exposed to a diversity of HA (H1, H3, and H4) and NA (N2, N6, N7, and N8) subtypes (Table 1). The raccoon yielding antibodies to the highest richness of influenza A virus subtypes was likely the oldest raccoon, based on tooth wear (the raccoon in question

had very worn and missing teeth), which was sampled in this study. This suggests that older and long-lived raccoons are more likely to be exposed to multiple subtypes of influenza A virus.

Overall, detected antibodies to influenza A virus persisted for at least 9 months. This is similar to a West Nile virus antibody study in rock pigeons (*Columba livia*), as West Nile virus antibodies were detectable for the duration of that study (15 months) (Gibbs et al. 2005). Our bELISA results revealed what appears to be a typical gradual decay of antibody titers over time. However, all raccoons maintained a detectable level of antibody for the duration of the study, thereby suggesting that antibodies in wild mammals may last for long periods, often without precipitous declines. Nonetheless, because these test animals were naturally exposed, in most instances (e.g., some were known to be exposed to more than one subtype) we have no way to determine if the antibodies we detected were from primary or secondary exposures, which may yield different responses in this species. Regardless, surveillance programs using antibodies to detect recent influenza A virus activity must take into account that these antibodies may portray a historical perspective in mammalian wildlife and only occasionally will represent recent exposures to influenza A virus. However, the longevity of these antibodies could be extremely useful to detect exposures months after outbreaks have occurred.

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